Molecular and Cellular Biology

# Overexpression of Glucose-6-Phosphate Dehydrogenase Is Associated with Lipid Dysregulation and Insulin Resistance in Obesity

**Jiyoung Park, Ho Kyung Rho, Kang Ho Kim, Sung Sik Choe, Yun Sok Lee and Jae Bum Kim**

***Mol. Cell. Biol.*** **2005, 25(12):5146. DOI: 10.1128/MCB.25.12.5146-5157.2005.**

MOLECULAR AND CELLULAR BIOLOGY, June 2005, p. 5146–5157 Vol. 25, No. 12
0270-7306/05/$08.00+0 doi:10.1128/MCB.25.12.5146–5157.2005




# Overexpression of Glucose-6-Phosphate Dehydrogenase Is Associated with Lipid Dysregulation and Insulin Resistance in Obesity†

Jiyoung Park, Ho Kyung Rho, Kang Ho Kim, Sung Sik Choe, Yun Sok Lee, and Jae Bum Kim*

*School of Biological Sciences, Seoul National University, Seoul 151-742, South Korea*

Received 16 September 2004/Returned for modification 20 October 2004/Accepted 11 March 2005

**Glucose-6-phosphate dehydrogenase (G6PD) produces cellular NADPH, which is required for the biosynthesis of fatty acids and cholesterol. Although G6PD is required for lipogenesis, it is poorly understood whether G6PD in adipocytes is involved in energy homeostasis, such as lipid and glucose metabolism. We report here that G6PD plays a role in adipogenesis and that its increase is tightly associated with the dysregulation of lipid metabolism and insulin resistance in obesity. We observed that the enzymatic activity and expression levels of G6PD were significantly elevated in white adipose tissues of obese models, including *db*/*db*, *ob*/*ob*, and diet-induced obesity mice. In 3T3-L1 cells, G6PD overexpression stimulated the expression of most adipocyte marker genes and elevated the levels of cellular free fatty acids, triglyceride, and FFA release. Consistently, G6PD knockdown via small interfering RNA attenuated adipocyte differentiation with less lipid droplet accumulation. Surprisingly, the expression of certain adipocytokines such as tumor necrosis factor alpha and resistin was increased, whereas that of adiponectin was decreased in G6PD overexpressed adipocytes. In accordance with these results, overexpression of G6PD impaired insulin signaling and suppressed insulin-dependent glucose uptake in adipocytes. Taken together, these data strongly suggest that aberrant increase of G6PD in obese and/or diabetic subjects would alter lipid metabolism and adipocytokine expression, thereby resulting in failure of lipid homeostasis and insulin resistance in adipocytes.**

Adipocytes are highly specialized cell type that maintains whole-body energy homeostasis by regulating glucose and lipid metabolism (60, 61). For a long time, adipocytes have been considered as an energy depot that stores and mobilizes triglyceride, a major energy fuel. Recently, adipocytes are also being recognized as an endocrine tissue with the discoveries of several adipocyte-secreted molecules, including lipid metabolites and adipocytokines (55). Using these molecules, adipocytes are able to communicate with other tissues and organs to regulate lipid and glucose metabolism, energy balance, insulin action, and reproduction.

To maintain lipid homeostasis, adipocytes carry out two reciprocal biochemical processes: lipogenesis and lipolysis. These two processes are tightly controlled by several hormones, lipid metabolites, and nutritional conditions such as feeding and fasting. With these signals, lipogenic transcription factors, including ADD1/SREBP1c, LXRs, and PPARγ actively participate in lipid metabolism in adipocytes. These transcription factors are highly expressed in adipose tissue and play a key role in adipocyte differentiation by coordinating lipogenic and adipocyte-specific gene expression (50). Because lipogenesis is tightly linked with adipogenesis, it is difficult to distinguish the two processes—lipogenesis and adipogenesis—during adipocyte differentiation.

Malonyl coenzyme A (malonyl-CoA) and NADPH are considered to be essential elements in lipogenesis (29, 54). Malonyl-CoA, which is produced by acetyl coenzyme A (acetyl-CoA) carboxylase (ACC), is a major substrate for long-chain fatty acid synthesis. It also suppresses fatty acid oxidation by inhibiting carnitine palmitoyltransferase (CPT-1) and acts as a signal molecule in appetite regulation (24, 52, 53, 66). Therefore, ACC2-null mice, with defects in malonyl-CoA synthesis, show a higher fatty acid oxidation rate and a significant loss of body fat mass (1, 2). NADPH also contributes to fatty acid synthesis by supplying reducing power. NADPH is produced by several enzymes, including malic enzyme (ME) and the first two enzymes of the pentose phosphate pathway (PPP), glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) (54). Although isocitrate dehydrogenase (IDH) also generates NADPH, its major function is irrelevant to lipogenesis (21, 54). Since NADPH is indispensable for the synthesis of fatty acids and cholesterol, it is plausible to speculate that NADPH producing enzymes might be associated with lipid metabolism disorders such as hyperlipidemia and lipid toxicity in metabolic diseases, including obesity and diabetes.

Among the four NADPH-producing enzymes, G6PD is the rate-limiting enzyme of PPP, and it is highly conserved in most mammalian species (33). G6PD plays a key role in the maintenance of redox potential and cell survival via production of NADPH and pentose phosphates (33). Previous researches on G6PD have been mostly focused on erythrocytes since G6PD deficiency in erythrocytes is a common human enzymopathy which results from a mutation in the G6PD gene (8). Other G6PD studies have been directed to cell growth, survival, and redox regulation with nonerythrocyte cells, including fibroblasts, hepatocytes, and neuronal cells (9, 33, 38, 54). G6PD also participates in reductive biosynthesis of fatty acids and cholesterol. Regarding the lipogenic activity of G6PD, it has been demonstrated that hepatic G6PD is regulated by nutri-

* Corresponding author. Mailing address: School of Biological Sciences, Seoul National University, San 56-1, Sillim-Dong, Kwanak-Gu, Seoul 151-742, South Korea. Phone: 82-2-880-5852. Fax: 82-2-878-5852. E-mail: jaebkim@snu.ac.kr.

† Supplemental material for this article may be found at http://mcb.asm.org/.

tional signals, including high-carbohydrate diet, polyunsaturated fatty acids, and hormonal signals such as insulin, glucagon, thyroid, and glucocorticoids (33, 54). Furthermore, it has been reported that G6PD-deficient patients show a decrease in lipogenic rate and serum lipoprotein concentrations, implying the importance of G6PD in fatty acid synthesis (15, 16). Nonetheless, the functional roles of G6PD in adipocytes are poorly understood.

In the present study, we demonstrated that both G6PD mRNA and protein were highly expressed in adipocytes, and their levels were significantly elevated in fat tissues of several obese mouse models. In adipocytes, G6PD overexpression stimulated the expression of adipocyte marker genes, as well as the elevation of cellular free fatty acid (FFA), and triglyceride (TG) and FFA release into the medium. In addition, expression levels of several adipocytokines, including tumor necrosis factor alpha (TNF-α), resistin, and adiponectin were sensitively modulated by G6PD, which would then influence insulin sensitivity in adipocytes. To determine more precisely the physiological roles of G6PD, we suppressed G6PD expression via small interfering RNA (siRNA). The resulting G6PD knockdown impaired normal adipocyte differentiation by decreasing both intracellular lipid accumulation and adipocyte marker gene expression. Therefore, these data suggest that G6PD functions to enhance both lipogenesis and adipogenesis and that high levels of G6PD in fat cells would lead to abnormal lipid metabolism and insulin resistance, which are frequently found in obese animals.

## MATERIALS AND METHODS

**Cell culture.** 3T3-L1 cells were grown to confluence in Dulbecco modified Eagle medium (DMEM) supplemented with 10% bovine calf serum (BCS; Gibco-BRL). Differentiation of 3T3-L1 cells was induced as described previously (30). Briefly, after 2 days of postconfluence, 3T3-L1 cells were incubated with DMEM containing 10% fetal bovine serum (FBS; Gibco-BRL), 3-isobutyl-1-methylxanthine (500 μM), dexamethasone (1 μM), and insulin (5 μg/ml) for 48 h. Culture medium was changed every other day with DMEM containing 10% FBS and insulin (5 μg/ml). 3T3-F442A cells were maintained in DMEM containing 10% BCS and were differentiated into adipocytes by addition of the medium with 10% FBS and insulin (5 μg/ml) when the cells were confluent (30).

**Northern blot analysis and real-time RT-PCR.** Total RNA was isolated with TRIzol reagent (Invitrogen/Life Technologies) according to the manufacturer's protocol. Each RNA was then denatured in formamide and formaldehyde, and separated by electrophoresis on formaldehyde-containing agarose gels. After electrophoresis, RNA was transferred to nylon membrane (Schleicher & Schuell), which was cross-linked with UV and hybridized with DNA probes. DNA probes were labeled by random priming method by using the Klenow fragment of DNA polymerase I (Takara) and [α-$^{32}$P]dCTP (Amersham-Pharmacia). cDNAs used as probes were those for G6PD, 6PGD, ME, IDH, ADD1/SREBP1c, FAS, peroxisome proliferator-activated receptor γ (PPARγ), C/EBPα, aP2, HSL, and 36B4. To normalize RNA loading, blots were hybridized with a cDNA probe for human acidic ribosomal protein, 36B4. For real-time reverse transcription-PCR (RT-PCR) analysis, cDNAs were synthesized with SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen). It was analyzed in a model iCycler Real-Time PCR Detection System (Bio-Rad) with following primer sets: G6PD, sense (5′-CGATGGCAGAGCAGGT-3′) and antisense (5′-GATCTGGTCCTCACG-3′); FAS, sense (5′-TGCTCCCAGCTGCAGGC-3′) and antisense (5′-GCCCGGTAGCTCTGGGTGTA-3′); PPARγ, sense (5′-TTGCTGAACGTGAAGCCCATCGAGG-3′) and antisense (5′-GTCCTTGTAGATCTCCTGGAGCAG-3′); aP2, sense (5′-CAAAATGTGTGATGCCTTTGTG-3′) and antisense (5′-CTCTTCCTTTGGCTCATGCC-3′); TNF-α, sense (5′-GCCACCACGCTCTTCTGCCT-3′) and antisense (5′-CTGATGGTGTGGGTGAGGAG-3′); resistin, sense (5′-CAGAAGGCACAGCAGTCTTG-3′) and antisense (5′-GACCGGAGGACATCAGACAT-3′); adiponectin, sense (5′-GGCAGGAAAGGAGAACCTGG-3′) and antisense (5′-GCCTTGTCCTTCTTGAAGAG-3′); GAPDH, sense (5′-TGCACCACCAACTGCTTAG-3′) and antisense (5′-GGATGCAGGGATGATGTTC-3′); and cyclophilin, sense (5′-CAGACGCCACTGTCGCTTT-3′) and antisense (5′-TGTCTTTGGAACTTTGTCTGCAA-3′).

**Western blot analysis.** White adipose tissues and adipocytes were lysed with TGN buffer (50 mM Tris [pH 7.5], 150 mM NaCl, 1% Tween 20, 0.2% NP-40, 1 mM phenylmethylsulfonyl fluoride, 100 mM NaF, 1 mM $Na_3VO_4$, 10 μg of aprotinin/ml, 2 μg of pepstatin A/ml, and 10 μg of leupeptin/ml). Total cell lysates were centrifuged at 12,000 rpm at 4°C for 15 min for the removal of fat debris. The protein concentration was determined by BCA assay kit (Pierce). Western blot analyses were conducted according to Amersham Life Science's protocol. The proteins were separated by electrophoresis on a sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Millipore). After the transfer, the membranes were blocked with nonfat milk containing PBST and were probed with primary antibodies against G6PD, PPARγ, glycogen synthase kinase 3β (GSK3β), phospho-GSK3β, Myc, Flag, adiponectin, IR, IRS-1, Akt, phospho-Akt, and phosphotyrosine (4G10). G6PD antibodies were purchased from Sigma Aldrich. PPARγ, Myc, and Flag antibodies were acquired from Santa Cruz Biotechnology. GSK3β and phospho-GSK3β were purchased from Transduction Laboratory. IR, IRS-1, Akt, phospho-Akt and 4G10 antibodies were purchased from Cell Signaling Technology. Lastly, mouse adiponectin antibodies were provided by KOMED (Seoul, Korea). The results were visualized with horseradish peroxidase-conjugated secondary antibodies (Sigma Aldrich) and enhanced chemiluminescence.

**G6PD enzyme assay.** G6PD enzyme activities were determined by measuring the rate NADPH production. Since 6PGD, the second enzyme of PPP, also produces NADPH, both 6PGD and total dehydrogenase activity (G6PD plus 6PGD) were measured separately as previously described (64). G6PD activity was calculated by subtracting the activity of 6PGD from the total enzyme activity. Glucose-6-phosphate, 6-phosphogluconate, and $NADP^+$ were obtained from Sigma Aldrich. Protein levels were determined for each sample by using a BCA assay kit (Pierce), and each enzyme activity was normalized by determining the protein concentration.

**Measurement of cellular lipid metabolites.** Cell contents of triglycerides and cholesterol in the 3T3-L1 cells were measured by using a TG and cholesterol assay kit (Sigma Aldrich). The amount of free fatty acids was determined with a NEFA assay kit (Roche). Each analysis was performed as recommended by the manufacturer.

**Retrovirus infection.** Retroviruses were constructed in pBabe vectors by using puromycin selectable markers. Viral constructs were transfected into BOSC cells by using calcium-phosphate transfection method (48). Cells were incubated in DMEM containing 10% FBS for 48 h. The cell culture medium was filtered through a 0.45-μm-pore-size filter, and the viral supernatant was used for infection of 3T3-L1 preadipocytes with Polybrene (4 μg/ml). The cells were infected for at least 12 h and allowed to recover for 24 h with fresh medium. The infected cells were selected with puromycin (1 to 5 μg/ml).

**Adenovirus infection.** G6PD adenovirus was produced by Neurogenex (Seoul, Korea). G6PD cDNA was in frame fused with Flag epitope tag in its N terminus. For adenovirus infection, 3T3-L1 adipocytes (at day 6 after differentiation) were incubated with serum-free DMEM, and various titers of adenovirus for 16 h at 37°C. Then, the culture medium was replaced with fresh medium. Each experiment was performed at 72 h after viral infection.

**siRNA for G6PD.** The sequences of the oligonucleotides used to create pSUPER-retro-siRNA-G6PD were designed from Oligoengine tools. Three sets of mouse G6PD siRNA oligonucleotides are positioned at ca. 279 to 297, 546 to 564, and 1,149 to 1,167 nucleotides downstream from the transcription start site of mouse G6PD cDNA. Three constructs were referred as pSUPER-retro-siRNA-G6PD-2i (ca. 279 to 297 nucleotides), −5i (ca. 546 to 564 nucleotides), and −11i (ca. 1,149 to 1,167 nucleotides), respectively. The siRNA sequences were as follows: G6PD-2i-sense (5′-GATCCCCGAAAGACCTAAGCTGGAGGTTCAAGAGACCTCCAGCTTAGGTCTTTCTTTTTGGAAA-3′), G6PD-2i-antisense (5′-AGCTTTTCCAAAAAGAAAGACCTAAGCTGGAGGTCTCTTGAACCTCCAGCTTAGGTCTTTCGGG-3′), G6PD-5i-sense (5′-GATCCCCCTGTCGAACCACATCTCCTTTCAAGAGAAGGAGATGTGGTTCGACAGTTTTTGGAAA-3′), G6PD-5i-antisense (5′-AGCTTTTCCAAAAACTGTCGAACCACATCTCCTTCTCTTGAAAGGAGATGTGGTTCGACAGGGG-3′), G6PD-11i-sense (5′-GATCCCCCAGTGCAAGCGTAATGAGCTTCAAGAGAGCTCATTACGCTTGCACTGTTTTTGGAAA-3′), and G6PD-11i-antisense (5′-AGCTTTTCCAAAAACAGTGCAAGCGTAATGAGCTCTCTTGAAGCTCATTACGCTTGCACTGGGG-3′). These oligonucleotides were annealed and then cloned into pSUPER-Retro vector (OligoEngine). The DNA constructs were used to produce G6PD siRNA retrovirus. siRNA experiments were performed as described by the manufacturer's protocols (OligoEngine).

**Generation of G6PD enzyme dead mutants.** Site-directed mutagenesis of mouse G6PD cDNA was performed by using the QuikChange kit (Stratagene) with the following mutagenic primers (mutated sites were underlined): G6PD-S188F(m2) (5′-CTGTCGAACCACATCTTCTCTCTTTTCCGTGACG-3′) and G6PD-K386E(m4) (5′-CTTCCACCAACAGTGCGAGCGTAATGAGCTGGTC-3′).

**Glucose uptake assay.** Insulin stimulated glucose uptake in 3T3-L1 adipocytes was determined by measuring [$^{14}$C]2-deoxy-glucose uptake as previously described (7). In short, adenovirus-infected 3T3-L1 adipocytes were incubated in low-glucose DMEM containing 0.1% bovine serum albumin for 16 h at 37°C. Cells were stimulated with or without 100 nM insulin for 1 h at 37°C. Glucose uptake was initiated by the addition of [$^{14}$C]2-deoxy-D-glucose at a final concentration of 3 μmol/liter for 10 min in HEPES buffer saline (140 mM NaCl, 5 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 20 mM HEPES [pH 7.4]). The reaction was terminated by separating cells from HEPES buffer saline and [$^{14}$C]2-deoxy-D-glucose. After three washes in ice-cold PBS, the cells were extracted by 0.1% SDS and subjected to scintillation counting for $^{14}$C radioactivity. The protein concentration was determined with a BCA assay kit (Pierce), and the radioactivities were normalized by determining each protein concentration.

## RESULTS

**Expression of G6PD mRNA in mouse tissues and adipocyte cell lines.** G6PD is known to be involved in lipogenesis by producing NADPH. However, it has not been thoroughly investigated whether adipose tissues and fat cells express G6PD. In order to examine the tissue distribution of G6PD mRNA, we performed Northern blot analysis. As shown in Fig. 1A, G6PD mRNA was highly expressed in adipose tissues. Also, kidneys, lungs, and spleens expressed moderate levels of G6PD mRNA (Fig. 1A). The mRNAs of 6PGD, ME, and IDH, other NADPH-producing enzymes, were abundantly expressed in adipose tissues, although their tissue distributions were not the same. Compared to preadipocytes such as 3T3-F442A and 3T3-L1, differentiated adipocytes prominently expressed G6PD mRNA, which was increased during adipogenesis (Fig. 1B). These observations implicate that G6PD might play important roles in lipogenesis or adipogenesis in fat cells.

**G6PD expression profiles in fat tissues of obese mice models.** Obese animal models, including *ob*/*ob*, *db*/*db*, and diet-induced obesity (DIO) mice, exhibit hyperlipidemia due to abnormal increases of lipid metabolism and fat tissues. However, the enzymes that contribute to this increase in lipogenic activity have not been clearly determined. Since G6PD is highly expressed in adipocytes and plays a role in lipogenesis, we decided to examine whether the level of G6PD expression is changed in the fat tissues of obese animals. To answer this question, we performed Northern blot analysis on subcutaneous and epididymal fat tissue of *db*/*db* mice. Surprisingly, the mRNA expression levels of G6PD were remarkably increased in both the subcutaneous (2.9-fold) and the epididymal (6.3-fold) fat pads of *db*/*db* mice compared to those of *db*/+ lean littermates (Fig. 2A and B). In contrast, the mRNA levels of other NADPH-producing enzymes, including IDH and ME, were either slightly changed or even decreased in *db*/*db* mice. Consistent with previous reports (43–45, 57–59), the mRNA levels of FAS and ADD1/SREBP1c were reduced in the fat tissues of *db*/*db* mice (Fig. 2A and B). We also investigated G6PD mRNA levels in other obese mice models such as *ob*/*ob* and DIO mice. Compared to lean mice, the mRNA levels of G6PD were clearly increased in both obese models (Fig. 2C). These results demonstrate that the level of G6PD mRNA is explicitly elevated in fat tissues of various obese mice models,

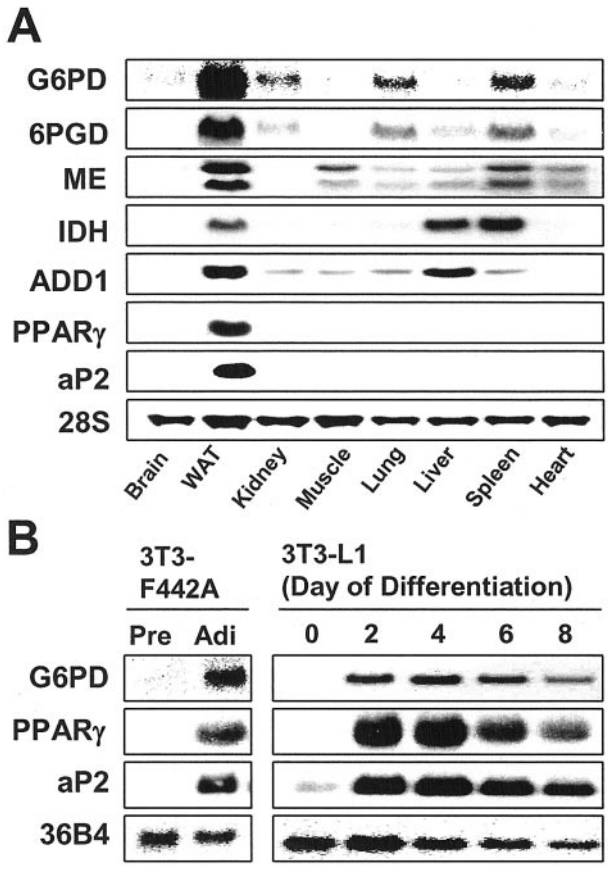


FIG. 1. Expression of G6PD mRNA in mouse tissues and adipocytes. (A) The indicated tissues were dissected from 10-week-old C57BL/6J mice. Total RNA was isolated, and mRNA levels of indicated genes were analyzed by Northern blot analyses. cDNA probes, including G6PD, 6PGD, ME, IDH, ADD1, PPARγ, and aP2, were used. (B) Total RNAs were isolated from 3T3-F442A and 3T3-L1 cells at the indicated time point after differentiation. Total RNA (20 μg) was analyzed for the expression of G6PD, PPARγ, and aP2 by Northern blot analyses. Equal loading was confirmed by assaying for 36B4. Pre, preadipocytes; Adi, adipocytes.

implying that the stimulation of G6PD expression appears to be associated with abnormal lipogenic activity in obese animals, leading to hyperlipidemia. Although more thorough investigations are required, we also observed that the expression level of G6PD mRNA in human fat tissues shows a tendency to increase with the degree of obesity (see Fig. S1 in the supplemental material).

To decide whether the protein levels and enzyme activity of G6PD are also elevated in the fat tissues of obese mice, we performed Western blot analysis and G6PD enzyme activity assays. The protein levels of G6PD were also increased (~3.3-fold) compared to lean mice (Fig. 3A). As expected from the mRNA and protein levels of G6PD, the enzymatic activity of G6PD was enhanced in various fat depots of *db*/*db* mice (Fig. 3B). However, G6PD enzymatic activities of the liver and muscle from *db*/*db* mice were insignificantly different from those of lean mice (Fig. 3B). Furthermore, the enzymatic activities of G6PD in fat tissues were at least 5- to 20-fold higher than that of liver in both normal and obese mice. Taken together, these

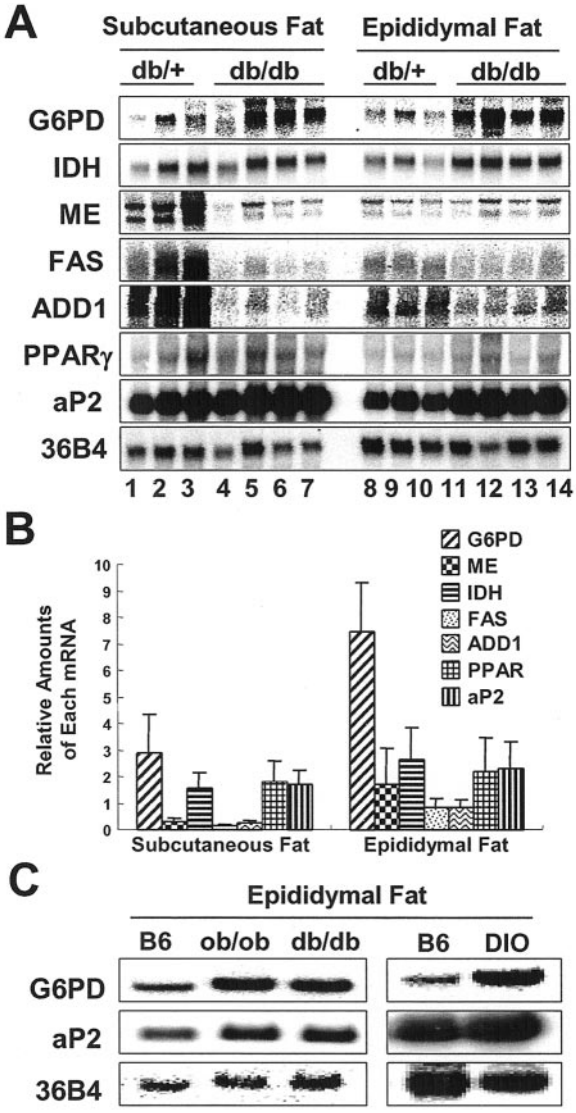


FIG. 2. Expression of G6PD mRNA in fat tissues of obese mice models, including *db*/*db*, *ob*/*ob* and diet-induced obesity (DIO). Subcutaneous or epididymal fat pads were dissected from 16-week-old *db*/+, *db*/*db*, C57BL/6J, *ob*/*ob*, and DIO (C57BL/6J mice on a high-fat diet for 9 weeks) mice. Total RNA was isolated, and the mRNA levels of indicated genes were represented by Northern blots. (A) cDNA probes including G6PD, ME, IDH, FAS, PPARγ, and aP2 were used, and equal loading was confirmed by assaying for 36B4. Subcutaneous fat, *db*/+ (lanes 1 to 3) and *db*/*db* (lanes 4 to 7); epididymal fat, *db*/+ (lanes 8 to 10) and *db*/*db* (lanes 11 to 14). (B) Relative amounts of each mRNA were quantified by measuring the ratio of each mRNA from *db*/*db* versus *db*/+ mice after normalization with 36B4, respectively. The intensity of each mRNA was determined by using a phosphorimager with Image-Master software (Pharmacia Biomedical Systems). (C) Representative Northern blots show the mRNA levels of G6PD and aP2. B6 stands for C57BL/6J. Equal loading was confirmed by assaying for 36B4.

observations suggest that both the expression level and the enzymatic activity of G6PD are remarkably elevated in several fat pads of obese animals, which may link to lipid dysregulation.

**Overexpression of G6PD stimulates adipogenesis and lipogenesis.** Next, to examine functional roles of G6PD in adipocytes, the effects of G6PD overexpression were investigated on the lipogenic and/or adipogenic potential. Retrovirus overexpression of G6PD in 3T3-L1 cells enhanced ~1.4-fold of its enzyme activity in comparison to mock retrovirus-infected adipocytes (Fig. 4A). On day 6 after the induction of adipocyte differentiation, G6PD-overexpressed 3T3-L1 cells showed enhanced adipocyte morphology with larger and more lipid droplet accumulation (Fig. 4B). Northern blot analyses were performed to investigate the change in lipogenic and adipogenic gene expression profiles. As shown in Fig. 4C, G6PD overexpression markedly promoted the expression of most adipocyte marker genes, including G6PD, FAS, ADD1/SREBP1c, PPARγ, and aP2. These observations suggest that G6PD overexpression is able to stimulate adipogenesis with lipogenesis by increasing both adipogenic and lipogenic gene expression.

Next, we determined the levels of lipid metabolites such as TG, cholesterol, and FFAs in the absence or presence of G6PD overexpression. In G6PD overexpressed adipocytes, cellular FFAs and TG levels were elevated by 2- and 1.7-fold, respectively (Fig. 4D and E). Interestingly, the level of FFAs released into the cultured medium was also increased by ~1.4-fold (Fig. 4F). In contrast, cellular cholesterol levels were not significantly changed (data not shown). These results indicate that the level of G6PD expression is closely associated with the levels of fatty acid metabolites, including TG and FFAs in adipocytes. This implies that aberrant increase of G6PD in obese subjects might promote circulating plasma FFA level,

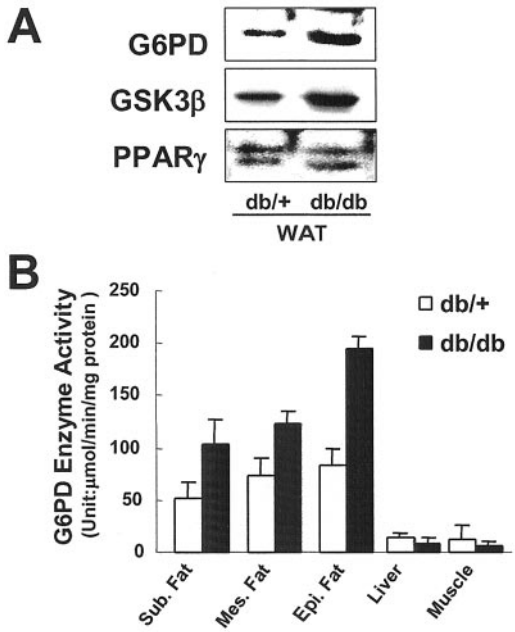


FIG. 3. Protein levels and enzymatic activities of G6PD in obese *db*/*db* mice tissues. (A) Total cell lysates were extracted from epididymal fat (white adipose tissues [WAT]) pads of *db*/+ and *db*/*db* mice. Immunoblotting was performed with G6PD, GSK3β, and PPARγ antibodies. (B) Subcutaneous (Sub.), mesenteric (Mes.), and epididymal (Epi.) fat pad, liver, and muscle tissues were dissected from *db*/+ and *db*/*db* mice. Tissue lysates were extracted and G6PD enzymatic activities were measured as described in Materials and Methods. The data are presented as mean ± the standard error (*db*/+ = three, *db*/*db* = four). The enzymatic activities were normalized by determining the protein concentrations.

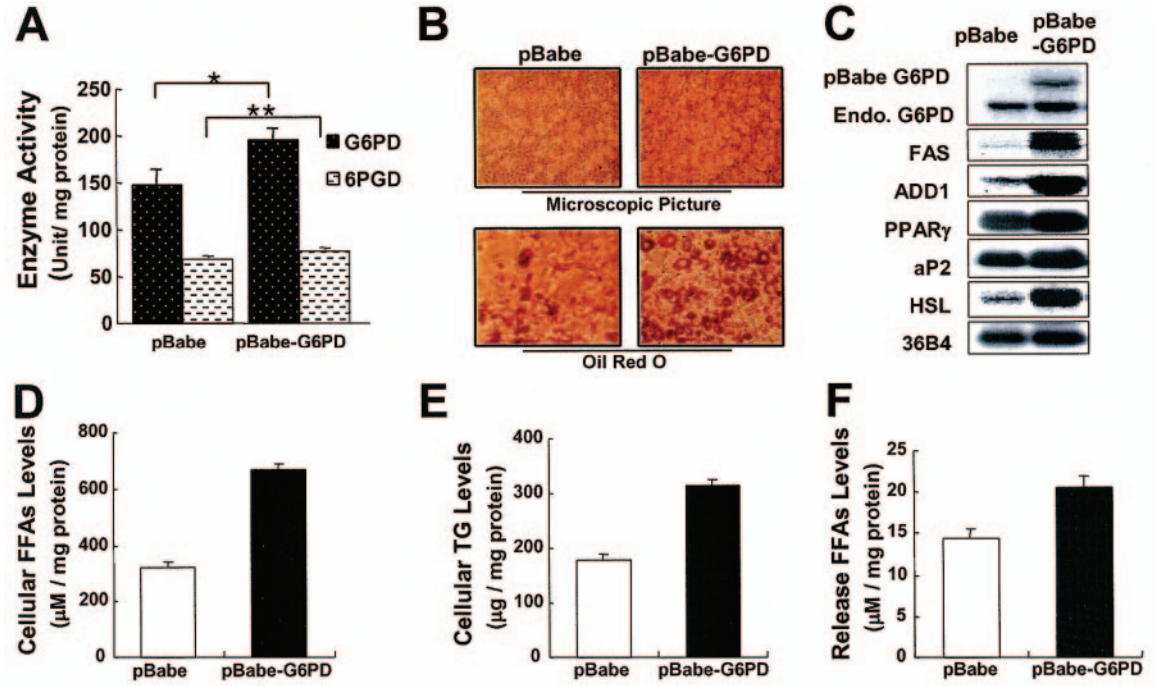


FIG. 4. Overexpression of G6PD stimulates adipogenesis in 3T3-L1 cells. 3T3-L1 preadipocytes were infected with mock (pBabe) or G6PD retrovirus (pBabe-G6PD), which were differentiated into mature adipocytes. (A) Proteins were extracted from pBabe or pBabe-G6PD-transduced 3T3-L1 adipocytes at 6 days after differentiation induction. G6PD and 6PGD enzymatic activities were measured as described in Materials and Methods. The data are presented as means ± standard errors ($n = 2$); ✻ and ✻✻, $P < 0.1$ and $P < 0.05$, respectively (versus pBabe and pBabe-G6PD). (B) Differentiated adipocytes were monitored by microscopic pictures with or without Oil Red O staining. (C) Northern blot analyses were performed to evaluate mRNA levels of G6PD, FAS, ADD1/SREBP1c, PPARγ, aP2, and HSL. Endo.G6PD stands for endogenous G6PD mRNA. (D to F) Total cell lysates were collected from pBabe or pBabe-G6PD overexpressed 3T3-L1 adipocytes. Cellular FFAs (D), TGs (E), and FFAs released into culture medium (F) were measured. The data were normalized by using protein concentrations. Each experiment was repeated three times, and data are presented as means ± standard errors ($n = 3$).

which is known to be a key cause of metabolic diseases, including insulin resistance and hyperlipidemia and lipotoxicity (3, 12, 13).

**Knockdown of G6PD impairs adipogenesis.** To verify whether G6PD is associated with adipocyte differentiation, we decided to knockdown G6PD by using siRNAs. We designed three different G6PD siRNA constructs into pSUPER retrovector (G6PD-2i, -5i, and -11i [see details in Materials and Methods]). We infected 3T3-L1 cells with these siRNA retroviruses and examined their abilities to suppress G6PD protein levels (Fig. 5A). Among these siRNAs, G6PD-11i most effectively suppressed the expression of endogenous G6PD protein (almost 90% reduction of G6PD protein), whereas G6PD-2i and G6PD-5i partially decreased G6PD protein in 3T3-L1 cells (Fig. 5A). Consistently, G6PD-11i effectively blunted G6PD enzyme activity (data not shown).

Next, we investigated the effect of G6PD knockdown on adipogenesis and lipogenesis. Consistent with our results that G6PD is involved in adipogenesis and lipogenesis (Fig. 4), 3T3-L1 cells infected with G6PD-11i-siRNA retrovirus showed attenuated adipocyte differentiation with a little lipid droplet accumulation (Fig. 5B). Furthermore, the cellular levels of TG and FFAs were significantly decreased in G6PD-11i-infected 3T3-L1 cells (Fig. 5C and D). Nonetheless, cellular cholesterol levels showed no significant change (Fig. 5E), which is also consistent with the results from G6PD-overexpressing cells.

To determine whether the reduction of G6PD expression might affect adipogenic gene expression in 3T3-L1 cells, we performed Northern blot analysis. As shown in Fig. 5F, the mRNA expression levels of most lipogenic genes including G6PD, 6PGD, ME, ADD1/SREBP1c, and FAS and those of adipogenic genes including PPARγ, C/EBPα, and aP2 were greatly decreased in G6PD-11i-infected 3T3-L1 cells, indicating that a certain amount of G6PD appears to be required for the execution of both adipogenesis and lipogenesis in adipocytes.

**Catalytic activity of G6PD is required for adipogenesis.** To clarify whether the effect of G6PD on adipogenesis is associated with its catalytic activity or scaffolding activity, we examined the effects of G6PD enzyme activity-deficient mutants on adipogenesis. On the basis of numerous studies from human G6PD mutants (8, 46), we generated two mutants, G6PD-S188F and G6PD-K386E (Fig. 6A). Mouse G6PD-S188F and K386E mutants were named G6PD-m2 and G6PD-m4, respectively. When G6PD-m2 or -m4 proteins were expressed to a similar extent compared with the wild type, the enzyme activities of both G6PD mutants were almost abolished in COS7 cells (Fig. 6B and C). To further investigate whether the catalytic activity of G6PD is required for adipogenesis, 3T3-L1 cells were infected with retroviruses containing G6PD-m2 or -m4 and were induced to differentiate into adipocytes for 6 days. As shown in Fig. 6D, G6PD-WT-overexpressing 3T3-L1

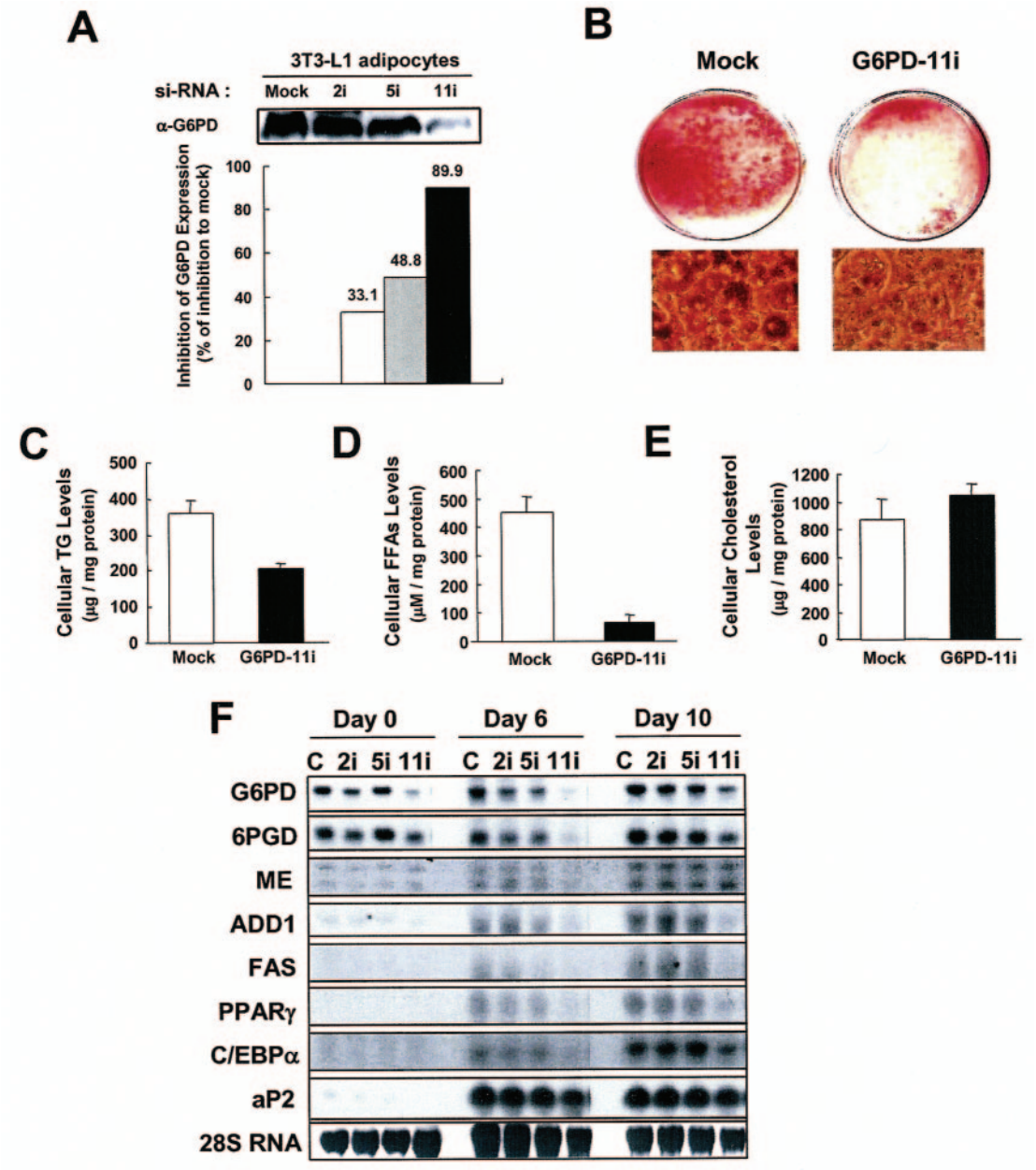


FIG. 5. Knockdown of G6PD suppresses lipogenic and adipogenic activities in 3T3-L1 adipocytes. Three synthetic siRNAs, which were targeting different regions of G6PD mRNAs (see details in Materials and Methods), were constructed into pSUPER retrovector. Retroviral siRNA constructs were used to infect 3T3-L1 cells, and the cells were differentiated into mature adipocytes. (A) Total cell lysates were collected from each retroviral siRNA-infected 3T3-L1 adipocytes (mock, G6PD-2i, -5i, and -11i). Immunoblotting with G6PD antibody indicates the protein levels of endogenous G6PD. (B) Mock or G6PD-11i siRNA-infected 3T3-L1 cells were induced into adipocytes, which were stained with Oil-Red O. Macroscopic (top) and microscopic (bottom) views of Oil-Red O staining are shown. (C to E) Total cell lysates were extracted from mock or G6PD-11i-infected 3T3-L1 adipocytes at 10 days after differentiation. Cellular TG levels (C), FFA levels (D), and cholesterol levels (E) were measured. The data are presented as means ± standard errors from three experiments. (F) Total RNAs were isolated from each retroviral siRNA (mock, G6PD-2i, -5i, and -11i)-infected cells at 0, 6, and 10 days after differentiation. Northern blot analyses were performed to evaluate mRNA levels of lipogenic (G6PD, 6PGD, ME, ADD1/SREBP1c, and FAS) and adipogenic (PPARγ, C/EBPα, and aP2) genes. Equal loading was confirmed by assessing levels of 28S rRNA.

cells enhanced adipogenesis, whereas G6PD-m2- and -m4-overexpressing cells were more or less differentiated into adipocytes than mock-infected cells. In addition, we measured G6PD enzymatic activity from wild-type and mutant G6PD-overexpressing 3T3-L1 adipocytes. As expected, 3T3-L1 cells overexpressing G6PD-m2 and -m4 exhibited lower G6PD activity than wild-type G6PD-overexpressing adipocytes (Fig. 6E). Consistent with these observations, adipogenic and lipogenic gene expression was not enhanced by overexpression of G6PD activity-deficient mutants (Fig. 6F). Therefore, it is

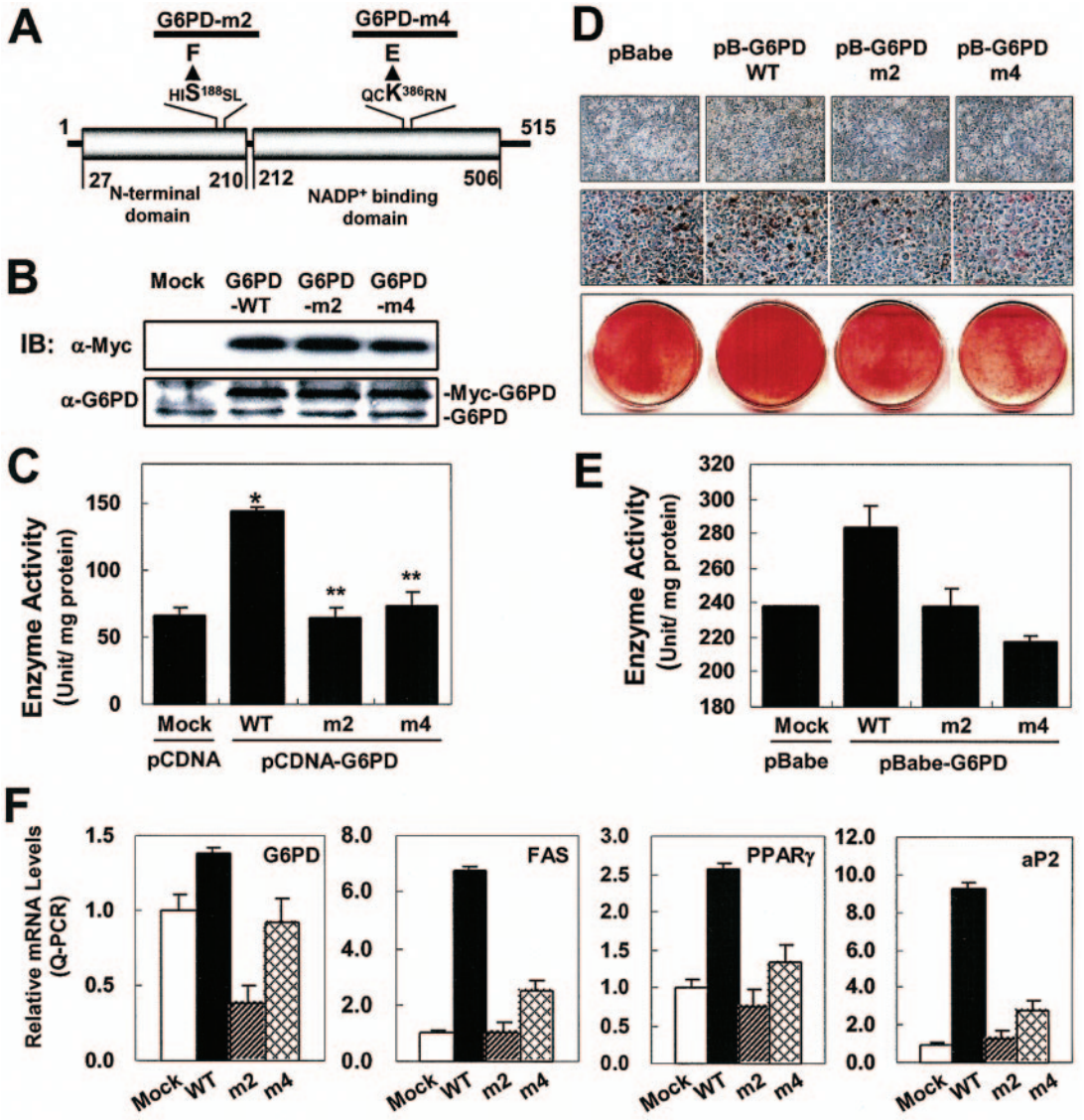


FIG. 6. Catalytic activity of G6PD is required for adipogenesis. (A) Schematic representation of mouse G6PD mutants: G6PD-m2 (S188F) and -m4 (K386E) were cloned into pCDNA 3.1(−)MycHis or pBabe vector. (B and C) After 2 days of transfection, the same amount of COS7 cells were lysed for Western blot and enzyme activity assay. (B) Immunoblotting of G6PD expression in COS7 cells with either vector alone or myc-tagged G6PD constructs (G6PD-WT, -m2, and -m4) with antibody to Myc epitope and G6PD. (C) G6PD enzymatic activities were measured as described in Materials and Methods. The data are presented as means ± standard errors ($n = 5$). *, $P < 0.01$ compared to mock-infected cells; **, $P < 0.05$ compared to G6PD-WT-infected cells. (D to F) 3T3-L1 cells were infected with retroviruses containing mock-, G6PD-WT-, -m2-, or -m4-infected cells and induced into adipocytes. (D) Differentiated adipocytes were monitored by analysis with or without Oil Red O staining. (E) G6PD activity was measured from wild-type and G6PD mutant overexpressing 3T3-L1 adipocytes. The data are presented as means ± standard errors ($n = 3$). (F) Each mRNA expression level of G6PD, FAS, PPARγ, and aP2 in mock-, G6PD-WT-, -m2-, or -m4-overexpressing 3T3-L1 adipocytes was measured by real-time RT-PCR. Values are normalized to the levels of GAPDH and cyclophilin and are represented as means ± standard errors ($n = 3$).

likely that G6PD stimulates adipogenesis through its enzymatic activity rather than by functioning as a scaffolding protein.

**G6PD overexpression changes the expression of adipocytokines.** It has been demonstrated that abnormal increase of cellular FFAs, TG, and FFA releases leads to the change of adipocytokine production and insulin sensitivity in adipocytes (3, 10, 13, 51). To examine the effect of G6PD overexpression on the expression or secretion of adipocytokines, we used G6PD adenovirus (AdG6PD) to infect differentiated adipocytes. Compared to mock adenovirus (AdMock)-infected adipocytes, AdG6PD-infected adipocytes (50 PFU/cell infection) expressed 1.5-fold more of G6PD protein (Fig. 7A). Accordingly, the enzyme activity of G6PD was increased by adenoviral G6PD expression (data not shown). Furthermore, adenoviral G6PD expression in adipocytes increased the release of FFAs into culture medium (Fig. 7B).

To determine the level of adipocytokine expression, we conducted real-time RT-PCR and Western blot analyses in the absence or presence of ectopic G6PD expression. Interestingly, infection with AdG6PD dramatically suppressed the protein

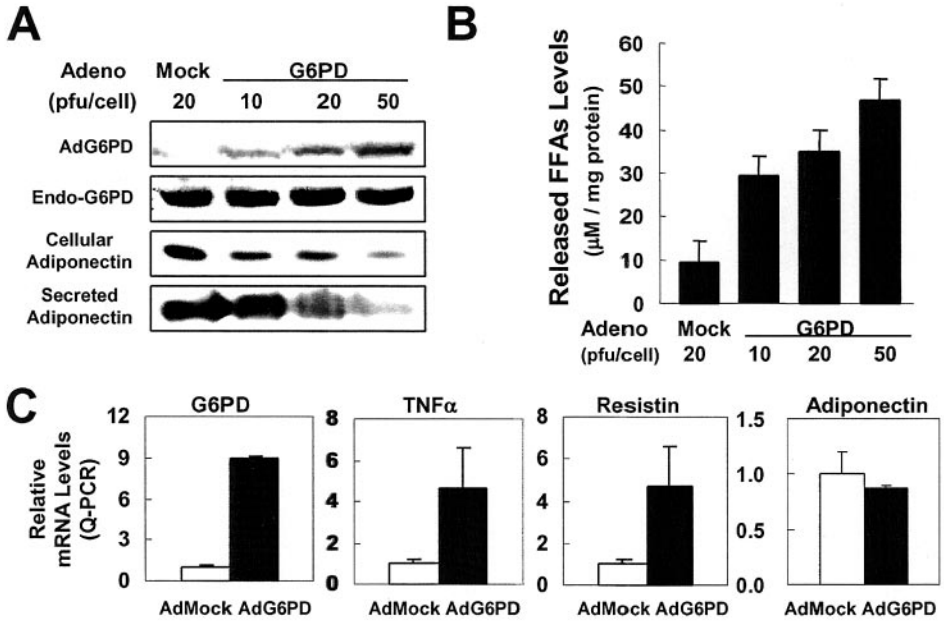


FIG. 7. Effects of G6PD overexpression on the expression of adipocytokines, including adiponectin, TNF-α, resistin, and FFAs. 3T3-L1 adipocytes were infected with different titers of AdG6PD (0 to 50 PFU/cell) for 16 h. (A) Total cell lysates were isolated from AdG6PD-infected 3T3-L1 adipocytes at 72 h after infection. Protein samples were separated by SDS-polyacrylamide gel electrophoresis, and immunoblotting was performed with antibodies to adiponectin, G6PD, and Flag, which detected only AdG6PD. (B) Released FFAs into culture medium were measured with a NEFA assay kit (Roche). Obtained results were normalized by protein concentrations. The data are presented as means ± standard errors ($n = 3$). (C) Total RNA was isolated from mock- or AdG6PD-infected adipocytes, and mRNA levels of indicated genes (G6PD, TNF-α, resistin, and adiponectin) were determined by real-time RT-PCR. Each mRNA amount was normalized with GAPDH and cyclophilin.

and mRNA expression of adiponectin (Fig. 7A and C). Normal expression of adiponectin is crucial to maintain insulin sensitivity and to prevent atherosclerosis (5, 39, 47, 70). Also, reduction of adiponectin level is found in obese or diabetic subjects (35, 41). Furthermore, real-time RT-PCR analyses showed that mRNA levels of TNF-α and resistin were greatly elevated in G6PD-overexpressed adipocytes (Fig. 7C). Among these adipocytokines, TNF-α and resistin are well known to induce insulin resistance in obese or diabetic animal models, whereas adiponectin enhances insulin sensitivity (41, 42). Therefore, these observations strongly suggest that increase of G6PD levels in adipocytes would alter insulin sensitivity not only by changing lipid metabolites but also by modulating adipocytokine expression.

**G6PD overexpression attenuates insulin signaling.** Since overexpression of G6PD in adipocytes increased the levels of FFAs, TNF-α, and resistin, which are key players in insulin resistance (Fig. 7), we hypothesized that G6PD overexpression would induce insulin resistance. To test this hypothesis, we examined the insulin signaling cascade with or without AdG6PD infection. AdMock-infected adipocytes exhibited increased tyrosine phosphorylation of insulin receptor (IR) and IR substrate 1 (IRS-1) and phosphorylation of Akt and GSKβ when treated with insulin (Fig. 8A and B). In contrast, these phosphorylation events were significantly diminished in AdG6PD-infected adipocytes (Fig. 8A and B).

It is well known that thiazolidinediones (TZDs), synthetic ligands of PPARγ, improve insulin sensitivity (20). Thus, we decided to examine whether the strong insulin sensitizer, TZD, is able to rescue insulin resistance in the presence of G6PD overexpressed adipocytes. As shown in Fig. 8, rosiglitazone, a member of TZD, clearly reversed the attenuated insulin sensitivity by G6PD overexpression (Fig. 8A and B). These results suggest that G6PD overexpression diminished insulin signaling, which is probably associated with change of adipocytokine expression, whereas TZD could reverse the insulin resistance induced by G6PD overexpression (Fig. 8A and B).

To confirm these results, we conducted insulin stimulated glucose uptake assay because it is a good measure of insulin sensitivity in adipocytes. We determined the ability of AdG6PD-infected 3T3-L1 adipocytes to uptake [$^{14}$C]2-deoxy-D-glucose in response to insulin. As shown in Fig. 8C, the fold increases in insulin-stimulated glucose uptake were reduced in G6PD-overexpressed adipocytes, and these responses were rescued by rosiglitazone treatment. Together, these results implicate that abnormal increase of G6PD expression in adipocytes would interfere with insulin signaling and thereby induce insulin resistance in obese subjects.

## DISCUSSION

In the past several decades, obesity has become a common health issue. Obesity is a major risk factor for metabolic diseases, including hyperlipidemia, hypercholesterolemia, cardiovascular disease, and type II diabetes (28, 36, 72). In many cases, metabolic diseases are closely associated with a failure of lipid homeostasis. A balance between fat synthesis (lipogenesis) and fat breakdown (lipolysis/fatty acid oxidation) is critical for the maintenance of lipid homeostasis that prevents lipotoxicity in the organs of overnourished individuals by confining

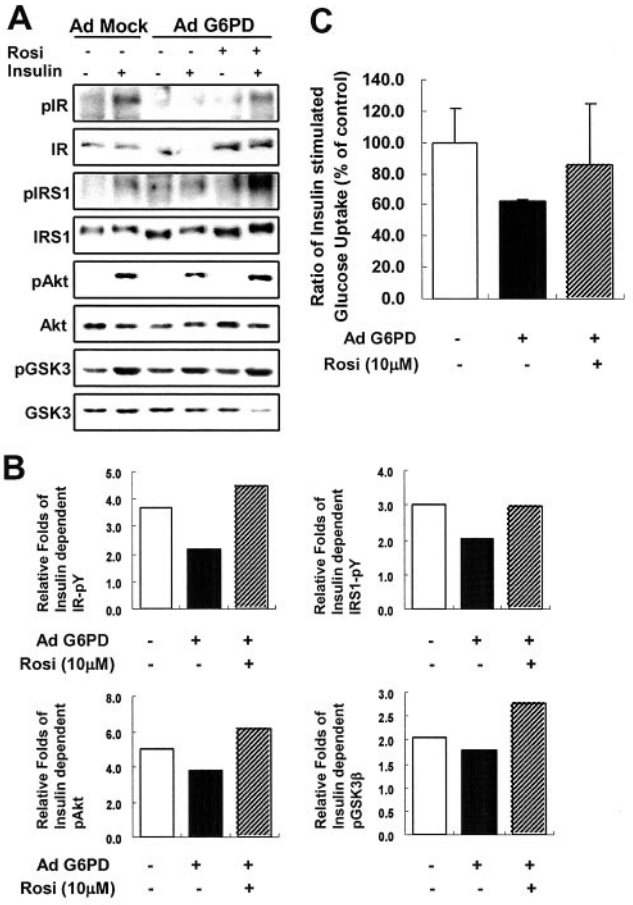


FIG. 8. Effect of G6PD overexpression on insulin signaling (A and B) and insulin-stimulated glucose uptake (C) in 3T3-L1 adipocytes. 3T3-L1 adipocytes were infected with mock or G6PD adenovirus. At 2 days after infection, cells were starved for 16 h with or without treatment of rosiglitazone (10 μM). (A) The cells were subsequently treated with 100 nM insulin at 37°C for 10 min, and the cell lysates were subjected to immunoblotting with anti-IR, anti-IRS1, antiphosphotyrosine (4G10), anti-Akt, anti-pAkt, anti-GSK3β, or anti-pGSK3β antibody. (B) Relative fold increases in insulin-stimulated phosphorylation of IR, IRS1, Akt, and GSK3β. The levels of each phosphoprotein were normalized with those of total protein amounts. (C) G6PD overexpression decreased insulin-stimulated [$^{14}$C]2-deoxyglucose uptake. AdMock- or AdG6PD-infected 3T3-L1 adipocytes were starved with serum-free DMEM for 12 h. Insulin-stimulated glucose uptake assays were conducted as described in Materials and Methods. The cpm results were normalized with protein concentrations. The fold increases in insulin-stimulated glucose uptake were normalized by using AdMock-infected adipocytes. The results are presented as a percentage of the control (mean ± standard error, $n = 2$). Rosi, rosiglitazone.

excess lipid into adipocytes. Thus, defects in liporegulation lead to hyperlipidemia or lipid toxicity found in obesity and type II diabetes (28, 67). Consequently, tremendous research is currently being devoted to the identification of molecular targets that behave as a "switch" that controls lipid metabolism and to the development of drugs that specifically regulate lipid metabolism. In spite of these efforts, molecular mechanisms underlying lipid metabolism disorders in adipocytes have not been clearly understood (14).

We suggest here that G6PD, one of the major NADPH-producing enzymes, influences fat cell differentiation and lipogenesis, as well as insulin resistance, in obese subjects. Surprisingly, G6PD was expressed most abundantly in fat tissues, and its mRNA increased during adipocyte differentiation (Fig. 1). Furthermore, the expression levels of G6PD mRNA and its enzymatic activity were significantly elevated in the white fat tissues of several obese animal models, including *db*/*db*, *ob*/*ob*, and DIO mice (Fig. 2 and 3). These observations strongly suggest that G6PD would play important roles in adipocytes, and its abnormal expression would be linked to lipid metabolism disorders frequently found in obese animals. We also demonstrated that ectopic expression of G6PD stimulated both adipogenesis and lipogenesis. Retrovirus overexpression of G6PD in 3T3-L1 cells elevated lipid metabolites, including TG and FFAs, and promoted mRNA expression of most adipocyte marker genes, including FAS, ADD1/SREBP1c, aP2, and PPARγ (Fig. 4). Consistent with these results, G6PD knockdown via siRNA system decreased cellular FFA and TG levels and diminished adipogenic and lipogenic gene expression (Fig. 5), indicating that proper expression of G6PD is required for adipogenesis as well as lipogenesis.

By producing NADPH, it appears that G6PD stimulates lipogenic activity in adipocytes. However, the molecular mechanism by which G6PD overexpression increases cellular FFAs and FFA release into medium is not understood. This might be mediated by the hormone-sensitive lipase (HSL), since HSL is responsible for hydrolysis of accumulated TGs into FFAs and glycerol in adipocytes. In fact, we observed that G6PD-overexpressed adipocytes significantly stimulated HSL expression (Fig. 4C). Furthermore, the fact that FFA levels in adipocytes were regulated by G6PD expression provides an important clue to the link between the high level of G6PD in the fat tissues of obese subjects and metabolic disorders. Many recent studies have suggested that overproduced FFAs derived from adipocytes cause insulin resistance and/or lipid toxicity, which are linked with metabolic diseases such as type II diabetes, cardiovascular disease, and dyslipidemia (3, 28, 60). More recently, it has been shown that intracellular lipid accumulation within skeletal muscle due to increase of circulating FFAs correlates with insulin resistance independent of obesity (11). Therefore, these results strongly suggest that an aberrant increase of G6PD in adipocytes would elevate lipogenic activity, thus overproducing FFAs and TGs, which might eventually accelerate the onset of metabolic disorders.

In spite of these findings, it is difficult to distinguish adipogenesis and lipogenesis during fat cell development because adipogenesis is tightly associated with lipogenesis in fat cells. Although the molecular mechanisms of G6PD in adipogenesis and lipogenesis are not well defined yet, there are several possibilities for how G6PD overexpression is able to enhance adipogenesis and lipogenesis. First, as a rate-limiting enzyme of PPP, G6PD could affect cell growth and differentiation by regulating the PPP process. Previously, it was reported that dehydroepiandrosterone, an inhibitor of G6PD, interferes with adipogenesis by reducing the NADPH/NADP$^+$ ratio (18, 56). Moreover, xylulose-5-phosphate, one of product of PPP, is well known to be associated with regulation of lipogenesis (27, 65). Thus, it is likely that overproduction of xylulose-5-phosphate by G6PD enhanced lipogenesis. Second, it is also possible that NADPH-dependent transcriptional regulators may stimulate adipogenesis or lipogenesis. Recently, NADH-dependent acti-

vators or histone deacetylases have been identified, and several target genes involved in the differentiation process are modulated by these NADH-dependent coregulators (17, 25, 37, 71). Lastly, it appears that G6PD could accelerate adipogenesis by promoting free fatty acid metabolism, which might eventually generate ligands of PPARγ, a master transcription factor for adipocyte differentiation. A similar idea has been demonstrated that overexpression of the active form of ADD1/SREBP1c, which coordinates most lipogenic genes, accelerates adipogenesis in a nonadipogenic cell line by producing endogenous PPARγ ligands (31).

As a major tissue for whole-body energy homeostasis, adipose tissue integrates both central and peripheral metabolic signals and orchestrates energy balance. Therefore, abnormal increase or decrease of adipose tissue often causes systemic disorders, also known as metabolic diseases. Adipose tissues are also actively involved in many physiological and pathological processes by expressing various adipocytokines, including leptin, TNF-α, resistin, adiponectin, interleukin-6 (IL-6), and FFAs (28, 40, 61). Therefore, adipose tissues locally influence the equilibrium between lipogenesis and lipolysis and systematically contribute to whole-body energy homeostasis by an endocrine mechanism. It is well known that certain adipocytokines are important in provoking the insulin resistance found in many obese and diabetic subjects. For example, the elevation in circulating inflammatory cytokines such as TNF-α and IL-6 mediates insulin resistance and is common in obesity (19, 22, 68). An increased level of TNF-α in obese states leads to insulin resistance via a number of mechanisms, including the decrease of insulin signaling or GLUT4 expression (26, 62). In addition, the newly identified resistin has been proposed to link obesity and insulin resistance (32, 63). Resistin expression is increased in obese animals and decreased by the antidiabetes drug, rosiglitazone (63). Thus, it has been suggested that TNF-α, IL-6, and resistin participate in the regulation of glucose metabolism and that high levels of their expression result in insulin resistance and type II diabetes (4, 41, 49, 68). Unlike these adipocytokines, adiponectin that regulates both glucose and lipid metabolism increases insulin sensitivity (6, 40), and its expression levels are inversely correlated with pathological conditions such as obesity, diabetes, or atherosclerosis (23, 35). In addition, many reports have shown that adiponectin treatment improves insulin resistance, atherosclerosis and lipid metabolism disorders in several animal models (5, 39, 47, 69, 70).

It is of interest to note that the levels of adipocytokines are explicitly changed in G6PD-overexpressed adipocytes. As shown in Fig. 7C, overexpression of G6PD in adipocytes elevated the expression of TNF-α and resistin mRNAs. In contrast, both protein and mRNA levels of adiponectin were decreased (Fig. 7A and C). The molecular mechanism(s) underlying the G6PD-dependent changes in adipocytokine expression remains to be elucidated. Nonetheless, the idea that anomalous increase of G6PD in adipocytes would induce insulin resistance was confirmed by the observation that G6PD-overexpressed adipocytes exhibited downregulated insulin signaling and insulin-dependent glucose uptake (Fig. 8). Taken together, these results strongly support the hypothesis that aberrantly high expression of G6PD in fat tissues of obese animals would result in metabolic disorders including hyperlipidemia and insulin resistance not only by increasing lipogenic activity but also by modulating adipocytokine expression.

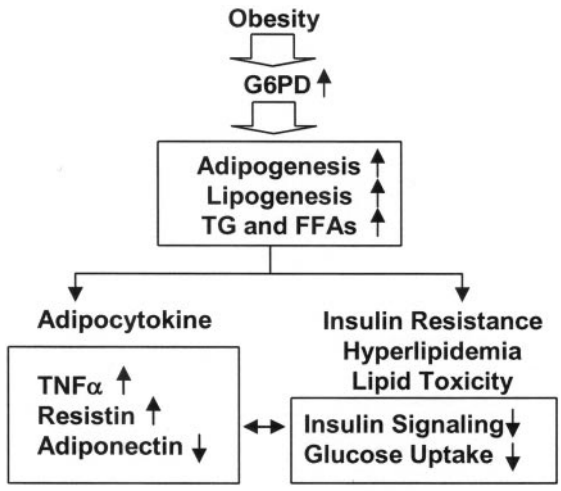


FIG. 9. Functional roles of G6PD in adipocytes and its impact on obesity. Obese subjects stimulate G6PD expression in adipocytes and enhance adiposity with elevation of TG and FFAs, which would affect the expression of adipocytokines and metabolic syndromes, including insulin resistance, hyperlipidemia, and lipotoxicity. See the text for further explanation.

Very recently, Koh et al. reported that IDPc is important for modulating fat and cholesterol synthesis as well as adipogenesis by showing that overexpression of IDPc increases 3T3-L1 adipocyte differentiation, whereas reduced expression of IDPc blocks adipogenesis (34). These results are somewhat similar to our observations that overexpression of G6PD in adipocytes promote lipogenesis and adipogenesis like IDPc overexpression, which could increase eventually cellular NADPH level. However, several evidences suggest that IDPc would have different physiological roles from G6PD. First, compared to G6PD, the mRNA level of IDPc is not significantly changed in the fat tissue of obese mice models (Fig. 2). Second, the mRNA level of G6PD was increased during adipocytes differentiation (Fig. 1), whereas that of IDPc is not changed (34). Furthermore, IDPc transgenic mice show lower fasting blood glucose levels and improvement of glucose sensitivity compared to wild-type mice, even though they exhibit obesity, hyperlipidemia, and fatty liver. In contrast, G6PD overexpression caused insulin resistance in adipocytes (Fig. 8). In accordance with this observation, G6PD overexpression increased the expression of TNF-α and resistin but decreased expression of adiponectin, which is closely related to a decrease in insulin sensitivity (Fig. 8). Thus, G6PD overexpression would lead to abnormal lipid metabolism and insulin signaling, which are different from IDPc overexpression.

In summary, we have established a novel link between G6PD and lipid metabolism disorders in the adipocytes of obese animals (Fig. 9). Our findings that G6PD expression is increased in obese animals and that G6PD elevation in adipocytes changes the levels of lipid metabolites provide strong evidence that obesity induced lipid metabolism disorders and insulin resistance is mediated, at least in part, by G6PD overexpression in fat tissues. Therefore, our observations that an abnor-

mal increase of G6PD in adipocytes modulates lipid metabolism and adipocytokine expression suggest a potential target in the successful development of therapeutic treatment for obese and diabetic patients.

## ACKNOWLEDGMENTS

We thank Thomas Lee and Jun-Jae Chung for critically reading the manuscript.

This study was supported in part by grants from Stem Cell Research Center of the 21st Century Frontier Research Program, the Molecular and Cellular Biodiscovery Research Program, and the National Research Laboratory Program of Korea Institute of Science and Technology Evaluation and Planning. J.P., H.K.R., K.H.K., S.S.C., Y.S.L., and J.B.K. are supported by the BK21 Research Fellowship from the Ministry of Education and Human Resources Development.

## REFERENCES

1. **Abu-Elheiga, L., M. M. Matzuk, K. A. Abo-Hashema, and S. J. Wakil.** 2001. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. Science **291:**2613–2616.
2. **Abu-Elheiga, L., W. Oh, P. Kordari, and S. J. Wakil.** 2003. Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. Proc. Natl. Acad. Sci. USA **100:** 10207–10212.
3. **Arner, P.** 2002. Insulin resistance in type 2 diabetes: role of fatty acids. Diabetes Metab. Res. Rev. **18**(Suppl. 2)**:**S5–S9.
4. **Banerjee, R. R., S. M. Rangwala, J. S. Shapiro, A. S. Rich, B. Rhoades, Y. Qi, J. Wang, M. W. Rajala, A. Pocai, P. E. Scherer, C. M. Steppan, R. S. Ahima, S. Obici, L. Rossetti, and M. A. Lazar.** 2004. Regulation of fasted blood glucose by resistin. Science **303:**1195–1198.
5. **Berg, A. H., T. P. Combs, X. Du, M. Brownlee, and P. E. Scherer.** 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat. Med. **7:**947–953.
6. **Berg, A. H., T. P. Combs, and P. E. Scherer.** 2002. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol. Metab. **13:**84–89.
7. **Berti, L., M. Kellerer, E. Capp, and H. U. Haring.** 1997. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. Diabetologia **40:**606–609.
8. **Beutler, E., and T. J. Vulliamy.** 2002. Hematologically important mutations: glucose-6-phosphate dehydrogenase. Blood Cells Mol. Dis. **28:**93–103.
9. **Biagiotti, E., L. Guidi, P. Del Grande, and P. Ninfali.** 2003. Glucose-6-phosphate dehydrogenase expression associated with NADPH-dependent reactions in cerebellar neurons. Cerebellum **2:**178–183.
10. **Boden, G.** 2001. Free fatty acids: the link between obesity and insulin resistance. Endocrinol. Pract. **7:**44–51.
11. **Boden, G.** 2002. Interaction between free fatty acids and glucose metabolism. Curr. Opin. Clin. Nutr. Metab. Care. **5:**545–549.
12. **Boden, G.** 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes **46:**3–10.
13. **Boden, G., and G. I. Shulman.** 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur. J. Clin. Investig. **32**(Suppl. 3)**:**14–23.
14. **Bray, G. A., and L. A. Tartaglia.** 2000. Medicinal strategies in the treatment of obesity. Nature **404:**672–677.
15. **Dessi, S., B. Batetta, O. Spano, D. Pulisci, M. F. Mulas, S. Muntoni, M. Armeni, C. Sanna, R. Antonucci, and P. Pani.** 1992. Serum lipoprotein pattern as modified in G6PD-deficient children during hemolytic anaemia induced by fava bean ingestion. Int. J. Exp. Pathol. **73:**157–160.
16. **Dessi, S., C. Chiodino, B. Batetta, A. M. Fadda, C. Anchisi, and P. Pani.** 1986. Hepatic glucose-6-phosphate dehydrogenase, cholesterogenesis, and serum lipoproteins in liver regeneration after partial hepatectomy. Exp. Mol. Pathol. **44:**169–176.
17. **Fulco, M., R. L. Schiltz, S. Iezzi, M. T. King, P. Zhao, Y. Kashiwaya, E. Hoffman, R. L. Veech, and V. Sartorelli.** 2003. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. Mol. Cell **12:**51–62.
18. **Gordon, G. B., J. A. Newitt, L. M. Shantz, D. E. Weng, and P. Talalay.** 1986. Inhibition of the conversion of 3T3 fibroblast clones to adipocytes by dehydroepiandrosterone and related anticarcinogenic steroids. Cancer Res. **46:** 3389–3395.
19. **Halle, M., A. Berg, H. Northoff, and J. Keul.** 1998. Importance of TNF-alpha and leptin in obesity and insulin resistance: a hypothesis on the impact of physical exercise. Exerc. Immunol. Rev. **4:**77–94.
20. **Hauner, H.** 2002. The mode of action of thiazolidinediones. Diabetes Metab. Res. Rev. **18**(Suppl. 2)**:**S10–S15.
21. **Horton, J. D., J. L. Goldstein, and M. S. Brown.** 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Investig. **109:**1125–1131.
22. **Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman.** 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science **259:**87–91.
23. **Hu, E., P. Liang, and B. M. Spiegelman.** 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J. Biol. Chem. **271:**10697–10703.
24. **Hu, Z., S. H. Cha, S. Chohnan, and M. D. Lane.** 2003. Hypothalamic malonyl-CoA as a mediator of feeding behavior. Proc. Natl. Acad. Sci. USA **100:**12624–12629.
25. **Imai, S., C. M. Armstrong, M. Kaeberlein, and L. Guarente.** 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:**795–800.
26. **Jain, R. G., K. D. Phelps, and P. H. Pekala.** 1999. Tumor necrosis factor-alpha initiated signal transduction in 3T3-L1 adipocytes. J. Cell Physiol. **179:** 58–66.
27. **Kabashima, T., T. Kawaguchi, B. E. Wadzinski, and K. Uyeda.** 2003. Xylulose 5-phosphate mediates glucose-induced lipogenesis by xylulose 5-phosphate-activated protein phosphatase in rat liver. Proc. Natl. Acad. Sci. USA **100:**5107–5112.
28. **Kahn, B. B., and J. S. Flier.** 2000. Obesity and insulin resistance. J. Clin. Investig. **106:**473–481.
29. **Kersten, S.** 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep. **2:**282–286.
30. **Kim, J. B., and B. M. Spiegelman.** 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. **10:**1096–1107.
31. **Kim, J. B., H. M. Wright, M. Wright, and B. M. Spiegelman.** 1998. ADD1/SREBP1 activates PPARγ through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA **95:**4333–4337.
32. **Kim, K. H., K. Lee, Y. S. Moon, and H. S. Sul.** 2001. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J. Biol. Chem. **276:**11252–11256.
33. **Kletzien, R. F., P. K. Harris, and L. A. Foellmi.** 1994. Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. FASEB J. **8:**174–181.
34. **Koh, H. J., S. M. Lee, B. G. Son, S. H. Lee, Z. Y. Ryoo, K. T. Chang, J. W. Park, D. C. Park, B. J. Song, R. L. Veech, H. Song, and T. L. Huh.** 2004. Cytosolic NADP+-dependent isocitrate dehydrogenase plays a key role in lipid metabolism. J. Biol. Chem. **279:**29968–29974.
35. **Kondo, H., I. Shimomura, Y. Matsukawa, M. Kumada, M. Takahashi, M. Matsuda, N. Ouchi, S. Kihara, T. Kawamoto, S. Sumitsuji, T. Funahashi, and Y. Matsuzawa.** 2002. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes **51:** 2325–2328.
36. **Kopelman, P. G.** 2000. Obesity as a medical problem. Nature **404:**635–643.
37. **Kumar, V., J. E. Carlson, K. A. Ohgi, T. A. Edwards, D. W. Rose, C. R. Escalante, M. G. Rosenfeld, and A. K. Aggarwal.** 2002. Transcription corepressor CtBP is an NAD(+)-regulated dehydrogenase. Mol. Cell **10:**857–869.
38. **Longo, L., O. C. Vanegas, M. Patel, V. Rosti, H. Li, J. Waka, T. Merghoub, P. P. Pandolfi, R. Notaro, K. Manova, and L. Luzzatto.** 2002. Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal. EMBO J. **21:**4229–4239.
39. **Matsuda, M., I. Shimomura, M. Sata, Y. Arita, M. Nishida, N. Maeda, M. Kumada, Y. Okamoto, H. Nagaretani, H. Nishizawa, K. Kishida, R. Komuro, N. Ouchi, S. Kihara, R. Nagai, T. Funahashi, and Y. Matsuzawa.** 2002. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J. Biol. Chem. **277:**37487–37491.
40. **Matsuzawa, Y., T. Funahashi, S. Kihara, and I. Shimomura.** 2004. Adiponectin and metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. **24:** 29–33.
41. **Matsuzawa, Y., T. Funahashi, and T. Nakamura.** 1999. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. Ann. N. Y. Acad. Sci. **892:**146–154.
42. **Mohamed-Ali, V., J. H. Pinkney, and S. W. Coppack.** 1998. Adipose tissue as an endocrine and paracrine organ. Int. J. Obesity Relat. Metab. Disorders **22:**1145–1158.
43. **Moitra, J., M. M. Mason, M. Olive, D. Krylov, O. Gavrilova, B. Marcus-Samuels, L. Feigenbaum, E. Lee, T. Aoyama, M. Eckhaus, M. L. Reitman, and C. Vinson.** 1998. Life without white fat: a transgenic mouse. Genes Dev. **12:**3168–3181.
44. **Nadler, S. T., and A. D. Attie.** 2001. Please pass the chips: genomic insights into obesity and diabetes. J. Nutr. **131:**2078–2081.
45. **Nadler, S. T., J. P. Stoehr, K. L. Schueler, G. Tanimoto, B. S. Yandell, and A. D. Attie.** 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. Proc. Natl. Acad. Sci. USA **97:**11371–11376.
46. **Naylor, C. E., P. Rowland, A. K. Basak, S. Gover, P. J. Mason, J. M. Bautista, T. J. Vulliamy, L. Luzzatto, and M. J. Adams.** 1996. Glucose 6-phosphate dehydrogenase mutations causing enzyme deficiency in a model of the tertiary structure of the human enzyme. Blood **87:**2974–2982.
47. **Okamoto, Y., S. Kihara, N. Ouchi, M. Nishida, Y. Arita, M. Kumada, K. Ohashi, N. Sakai, I. Shimomura, H. Kobayashi, N. Terasaka, T. Inaba, T.**

**Funahashi, and Y. Matsuzawa.** 2002. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation **106:**2767–2770.
48. **Pear, W. S., G. P. Nolan, M. L. Scott, and D. Baltimore.** 1993. Production of high-titer helper-free retroviruses by transient transfection. Proc. Natl. Acad. Sci. USA **90:**8392–8396.
49. **Rajala, M. W., and P. E. Scherer.** 2003. Minireview: the adipocyte–at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology **144:**3765–3773.
50. **Rosen, E. D., C. J. Walkey, P. Puigserver, and B. M. Spiegelman.** 2000. Transcriptional regulation of adipogenesis. Genes Dev. **14:**1293–1307.
51. **Ruan, H., and H. F. Lodish.** 2003. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. Cytokine Growth Factor Rev. **14:**447–455.
52. **Ruderman, N., and M. Prentki.** 2004. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. Nat. Rev. Drug Discov. **3:**340–351.
53. **Ruderman, N. B., A. K. Saha, and E. W. Kraegen.** 2003. Minireview: malonyl CoA, AMP-activated protein kinase, and adiposity. Endocrinology **144:** 5166–5171.
54. **Salati, L. M., and B. Amir-Ahmady.** 2001. Dietary regulation of expression of glucose-6-phosphate dehydrogenase. Annu. Rev. Nutr. **21:**121–140.
55. **Saltiel, A. R.** 2001. You are what you secrete. Nat. Med. **7:**887–888.
56. **Shantz, L. M., P. Talalay, and G. B. Gordon.** 1989. Mechanism of inhibition of growth of 3T3-L1 fibroblasts and their differentiation to adipocytes by dehydroepiandrosterone and related steroids: role of glucose-6-phosphate dehydrogenase. Proc. Natl. Acad. Sci. USA **86:**3852–3856.
57. **Shimomura, I., Y. Bashmakov, and J. D. Horton.** 1999. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J. Biol. Chem. **274:**30028–30032.
58. **Shimomura, I., R. E. Hammer, J. A. Richardson, S. Ikemoto, Y. Bashmakov, J. L. Goldstein, and M. S. Brown.** 1998. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev. **12:**3182–3194.
59. **Soukas, A., P. Cohen, N. D. Socci, and J. M. Friedman.** 2000. Leptin-specific patterns of gene expression in white adipose tissue. Genes Dev. **14:**963–980.
60. **Spiegelman, B. M., and J. S. Flier.** 1996. Adipogenesis and obesity: rounding out the big picture. Cell **87:**377–389.
61. **Spiegelman, B. M., and J. S. Flier.** 2001. Obesity and the regulation of energy balance. Cell **104:**531–543.
62. **Stephens, J. M., J. Lee, and P. F. Pilch.** 1997. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. J. Biol. Chem. **272:**971–976.
63. **Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar.** 2001. The hormone resistin links obesity to diabetes. Nature **409:**307–312.
64. **Tian, W. N., L. D. Braunstein, J. Pang, K. M. Stuhlmeier, Q. C. Xi, X. Tian, and R. C. Stanton.** 1998. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. J. Biol. Chem. **273:**10609–10617.
65. **Towle, H. C., E. N. Kaytor, and H. M. Shih.** 1997. Regulation of the expression of lipogenic enzyme genes by carbohydrate. Annu. Rev. Nutr. **17:**405–433.
66. **Unger, R. H.** 2004. The hyperleptinemia of obesity-regulator of caloric surpluses. Cell **117:**145–146.
67. **Unger, R. H.** 2003. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology **144:**5159–5165.
68. **Wellen, K. E., and G. S. Hotamisligil.** 2003. Obesity-induced inflammatory changes in adipose tissue. J. Clin. Investig. **112:**1785–1788.
69. **Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn, and T. Kadowaki.** 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. **8:**1288–1295.
70. **Yamauchi, T., J. Kamon, H. Waki, Y. Imai, N. Shimozawa, K. Hioki, S. Uchida, Y. Ito, K. Takakuwa, J. Matsui, M. Takata, K. Eto, Y. Terauchi, K. Komeda, M. Tsunoda, K. Murakami, Y. Ohnishi, T. Naitoh, K. Yamamura, Y. Ueyama, P. Froguel, S. Kimura, R. Nagai, and T. Kadowaki.** 2003. Globular adiponectin protected *ob*/*ob* mice from diabetes and ApoE-deficient mice from atherosclerosis. J. Biol. Chem. **278:**2461–2468.
71. **Zhang, Q., D. W. Piston, and R. H. Goodman.** 2002. Regulation of corepressor function by nuclear NADH. Science **295:**1895–1897.
72. **Zimmet, P., K. G. Alberti, and J. Shaw.** 2001. Global and societal implications of the diabetes epidemic. Nature **414:**782–787.